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β-Thalassaemia in Cubans: Novel Allele Increases the Genetic Diversity at the *HBB* Locus in the Caribbean

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In order to establish the molecular basis of β-thalassaemia in Cubans, a total of 75 unrelated individuals, with β-thalassaemia major (7), Hb S-β-thalassaemia (28), Hb C-β-thalassaemia (1), and β-thalassaemia trait (39) yielding 82 β-thalassaemia alleles, were analyzed. Seventeen different point mutations were identified accounting for 93% of the β-thalassaemia alleles studied, revealing a high genetic heterogeneity at the *HBB* locus in this population. The more prevalent mutations, namely, CD 39 (C → T) (30.5%), −29 (A → G) (13.4%), IVS-I-110 (G → A) (8.5%), and IVS-II-1 (G → A) (8.5%), reflect the Mediterranean and African predominant ancestry of the extant Cuban population. We also report the identification of a novel allele, IVS-I-108 (T → C), that possibly activates a cryptic branch site, in a β-thalassaemia carrier with no other molecular defect within the β-globin gene and its proximal promoter. This study shows that prenatal diagnosis of β-thalassaemia should be feasible in about 60% of at-risk pregnancies by direct detection of selected point mutations. However, due to the wide spectrum of mutations, and in order to offer fully informative prenatal diagnosis to more than 87% of at-risk couples, the screening for β-thalassaemia mutations in Cubans should be performed by using a general point mutation detection method, such as DGGE (denaturing gradient gel electrophoresis). Am. J. Hematol. 64:7–14, 2000.

Key words: β-thalassaemia mutations; cryptic branch site; prenatal diagnosis; Cubans

INTRODUCTION

The thalassaemias result from a wide variety of different mutations of the α- and β-globin genes that direct synthesis of adult haemoglobin ($\alpha_2\beta_2$). Imbalanced globin chain ratio is a major factor in determining the severity of anaemia in β-thalassaemia (β-thal) [1].

Over 186 different β-thal mutations have been identified so far [2]. Most of these molecular defects are single base substitutions or small insertions or deletions within or flanking the β-globin gene. In general, each population presents a group of few common β-thal mutations and a larger number of rare ones [1].

Cuba is the largest island (110,860 km^2) in the Caribbean Sea with a population of about 11 million inhabitants. The present Cuban population originated mainly from Caucasian Spanish and sub-Saharan Africans: about 66% is made up of whites, 12% are blacks, and 21.9% are of mixed heritage. The remaining 0.1% are of Chinese origin [3]. Amerindian contribution is negligible since aborigenes were almost completely exterminated soon after the Spanish conquest beginning in 1510 [4].

The epidemiology of the genetic determinants of haemoglobin disorders found in the current Cuban population reflects its ancestry: the frequency of α^+ thalassaemia is 22.7% (in nonwhite Cubans) [5], β^S and β^C occur with gene frequencies of 1.5% and 0.35% respectively [6], whereas the frequency of β-thal alleles has been estimated to be 0.45% [7]. This epidemiological situation has prompted the organisation of a successful sickle-cell disease prevention program [8]. On the contrary, until now no effort has been made to elucidate the molecular basis of β-thal in the Cuban population, a prerequisite to set up a comparable prevention program.

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In this context, we describe here the spectrum and distribution of β-thal mutations among Cubans. A strategy for prenatal diagnosis of β-thal and Hb S-β-thal is also proposed.

MATERIALS AND METHODS

Subjects

Seventy-five unrelated individuals with β-thal major (7), Hb S-β-thal (28), Hb C-β-thal (1), and β-thal trait (39), yielding 82 β-thal alleles were studied. A subjective criterion of ethnic group ascertainment was applied dividing this population sample into three subpopulations: Cubans with an European origin (European-Cubans), Cubans with an African origin (African-Cubans), and Cubans with an Asian origin (Asian-Cubans). The latter group includes three Asian/European and two Asian/African *mestizos.*

Haematological Analysis

Peripheral blood samples were collected with EDTA as anticoagulant. The differential diagnosis of β-thal trait was based on elevated Hb A_2 levels (≥3.5%), determined by DEAE-cellulose chromatography [9], and elevated osmotic resistance [10]. Electrophoretic haemoglobin separation was performed on hydrolysed starch at pH 8.6 [11]. Hb F was determined by the Betke alkali denaturation method [12]. Family studies were performed whenever possible. Red blood cell indices were not determined in all cases.

DNA Analysis

Total genomic DNA was isolated from peripheral leukocytes by a standard method [13]. Four β-thal mutations common in Mediterranean populations, i.e., CD 39 (C → T), IVS-I-1 (G → A), IVS-I-6 (T → C), and IVS-I-110 (G → A), were screened by ARMS [14]. The screening for other point mutations in the β-globin gene was carried out by denaturing gradient gel electrophoresis (DGGE) [15]. Samples with an altered DGGE pattern were sequenced on double-stranded DNA by the dideoxy termination method [16]. In three samples with a normal DGGE pattern for all gene fragments, complete sequencing of IVS-II as well as the screening for the Asian Indian 619 bp deletion were performed [17]. Three β-thal major samples were not completely genotyped due to lack of DNA, each one of them having only one mutation identified.

The Shapiro–Senapathy Consensus Values

Consensus values (CVs), as defined by Shapiro and Senapathy [18], were calculated for the normal (CVN_{-37}) and the potentially activated cryptic branch sites due to IVS-I-108 (T → C) mutation (CVM_{-24} and CVM_{-22}), as well as for the already described cryptic branch sites with the same branch point (CVC_{-24} and CVC_{-22}). CVs were calculated by scoring nucleotides spanning positions −5 to +1 and −5 to +15 with respect to the branch point nucleotide (taken to be at position 0). The scores were obtained by comparing the sequence under analysis with the corresponding nucleotide frequency in a sample of 31 experimentally characterized branch sites [19]. It should be noted that 29 out of these 31 branch sites are of mammalian origin and 16 of them are from genes of foetal and adult haemoglobin chains (including some mutant sites). The potential for cryptic branch site utilisation (PCU) was calculated by the CV ratios (CVM_{-24}: CVN_{-37}), (CVC_{-24}: CVN_{-37}), and (CVM_{-22}: CVC_{-22}) [20].

Statistical Analysis

The chi-square (χ^2) statistics was used to assess difference significance in β-thal allele distribution, between the Cuban and other related populations, and within Cuban subpopulations.

RESULTS

DNA Analysis

We have elucidated the molecular basis of β-thal in 76 out of 82 alleles. Seventeen different point mutations were identified accounting for 93% of the studied β-thal alleles. Table I lists the different β-thal alleles observed in the Cuban population as a whole and in the European-Cuban, African-Cuban, and Asian-Cuban subpopulations.

The four more prevalent mutations, CD 39 (C → T) (30.5%), −29 (A → G) (13.4%), IVS-I-110 (G → A) (8.5%), and IVS-II-1 (G → A) (8.5%), account for only 61% of the molecular defects.

In our sample, six β-thal alleles (7.3%) were not identified: two in β-thal carriers, one in a Hb S-β-thal patient and three belonging to three β-thal major patients (Table II). In the first three individuals, complete β-globin gene analysis was performed, whereas in the last three this was not possible due to lack of DNA.

A number of mutations were predominantly found in the expected Cuban subpopulation: CD 39 (C → T), IVS-I-110 (G → A), and IVSI-1 (G → A) in European-Cubans; and −29 (A → G), IVS-II-1 (G → A), poly A (T → C), and −88 (C → T) in African-Cubans. The mutation CD 41/42 (−TTCT) was exclusively found in individuals with an Asian ancestry. On the contrary, the Mediterranean mutation CD 39 (C → T) accounts for 21% of the alleles in the African subpopulation and the African mutation poly A (T → C) was also detected in the European subpopulation (Table I). Among the patients studied, seven were diagnosed with β-thal major. All of them were compound heterozygotes, and their genotypes did not always correlate with their apparent ancestries (patients 2, 5 and 7), as also observed in the

TABLE I. Spectrum and Relative Abundance of β-Thal Mutations in the Cuban Population

Mutation	Type	First described in[a]	Allele Frequency (*n* (%)) European-Cubans	African-Cubans	Asian-Cubans	Cubans (Total)
CD 39 (C → T)	β°	Med	17 (47.2)	8 (21.1)	0	25 (30.5)
−29 (A → G)	β^+	Af./Chin	0	10 (26.3)	1 (12.5)	11 (13.4)
IVS-I-110 (G → A)	β^+	Med	4 (11.1)	3 (7.9)	0	7 (8.5)
IVS-II-1 (G → A)	β°	Af/Med	1 (2.8)	6 (15.8)	0	7 (8.5)
CD 41/42 (−TTCT)	β°	Chin	0	0	5 (62.5)	5 (6.1)
IVS-I-1 (G → A)	β°	Med	4 (11.1)	0	0	4 (4.9)
IVS-I-6 (T → C)	β^+	Med	2 (5.6)	1 (2.6)	0	3 (3.7)
poly A (T → C)	β^+	Af	1 (2.8)	2 (5.3)	0	3 (3.7)
−88 (C → T)	β^+	Af	0	2 (5.3)	0	2 (2.4)
CD 121 (G → T)	β°	Eur	2 (5.6)	0	0	2 (2.4)
initiations CD (ATG → ACG)	β°	Med	0	1 (2.6)	0	1 (1.2)
CD 14/15 (+G)	β°	Chin	0	0	1 (12.5)	1 (1.2)
CD 15 (TGG → TGA)	β°	Med	1 (2.8)	0	0	1 (1.2)
IVS-I, -1 (G → A) (CD 30)	?	Eur	0	1 (2.6)	0	1 (1.2)
IVS-I-5 (G →A)	β^+	Med	0	1 (2.6)	0	1 (1.2)
IVS-I-108 (T → C)	?	Novel	1 (2.8)	0	0	1 (1.2)
IVS-II-849 (AG)	β°	Af	0	1 (2.6)	0	1 (1.2)
Identified mutations			33 (91.7)	36 (94.7)	7 (87.5)	76 (92.7)
Nonidentified mutations			3 (8.3)	2 (5.3)	1 (12.5)	6 (7.3)
Total			36 (100.0)	38 (100.0)	8 (100.0)	82 (100.0)

[a]Med, Mediterraneans; Af, African-Americans; Eur, Europeans; Chin, Chinese.

other groups of patients (Table II). Nevertheless, a strong association between the Cuban subpopulations and β-thal mutation type (Mediterranean-European, African-American, Asian) was observed, with a concordance value of 76%.

Novel Mutation

We have identified a novel allele, IVS-I-108 (T → C), in a β-thal carrier (Hb A_2 = 3.6%) of European origin, with no other detectable molecular defect within the β-globin gene and its proximal promoter. This nucleotide change detected by DGGE analysis and sequenced in both senses (Fig. 1) was not found in 64 normal β-globin genes.

This mutation does not create the invariant AG dinucleotide present in all described acceptor splice sites [21] as in the neighboring IVS-I-110 (G → A) and IVS-I-116 (T → G) β-thal mutations. Nevertheless, its location 23 nucleotides upstream from the 3′ splice junction, in close vicinity to the branch site CACUGAC, with the A for the 5′–2′ bond (branch point) located at position −37, possibly affects splicing efficiency [22–24].

Splicing of pre-mRNA introns occurs within the spliceosome, which contains U1, U2, U5, and U4/U6 small nuclear ribonuclear proteins (snRNPs) in addition to a large number of splicing factors [21]. The sequences required for splicing include the conserved donor 5′-splice site and three associated elements at the intron 3′ end, namely the conserved acceptor 3′-splice site, a polypyrimidine stretch, and the less conserved branch point sequence at the site of lariat formation [22]. Although the branch site is not completely conserved in higher eukaryotes, a consensus sequence has been proposed due to a clear preference for purines or pyrimidines at each position: Py_{72} N Py_{86} Py_{72} Pu_{69} A_{86} Py_{69} [19].

Mutations in the functional branch site of the human β-globin IVS-I lead to the activation of cryptic branch points in vitro, located 22 to 37 nucleotides upstream the 3′ splice site, which allow normal splicing, albeit at a reduced rate. An A →G transition of the branch point in this intron results in the activation of a cryptic branch site with the branch point at position −24 with a 5-fold reduction of the splicing efficiency [25]. Branch point analyses performed during the course of in vitro splicing assays of a normal human β-globin pre-mRNA revealed that functional branches are formed, at an early stage, on U residues at positions −17 and −22, in addition to the authentic one using A at position −37 [26].

We hypothesize that the novel mutation, IVS-I-108 (T → C), located 23 nt upstream the IVS-I/exon 2 junction, may activate a cryptic branch site (Table III): (i) the mutation originates the sequence UGCCUAC (with the putative branch point A at position −24) which has only one mismatch when compared with the consensus sequence, and its similarity with the normally functional branch site (with the branch point A at position −37) is larger than that of the previously described cryptic branch sites; (ii) a less probable hypothesis, could be the

TABLE II. Distribution of Cuban Individuals With Different β-Thal Phenotypes According to Their Ancestries and β-Thal Mutations

β-Thal phenotype	No. of individuals	Ancestry	β-Thal mutations	*n*[a]
β-Thal trait	27	European	CD 39 (C → T)	12
			IVSI-1 (G → A)	4
			IVS-I-110 (G → A)	4
			CD121 (G → T)	2
			IVSI-6 (T → C)	1
			IVS-II-1 (G → A)	1
			CD 15 (TGG → TGA)	1
			IVS-I-108 (T → C)	1[b]
			Nonidentified mutation	1
	9	African	CD 39 (C → T)	4
			−29 (A → G)	2
			IVSI-6 (T → C)	1
			IVS-II-849 (A → G)	1
			Nonidentified mutation	1
	3	Asian	CD 41/42 (−TTCT)	3
Hb S-β-thal	5	European	CD 39 (C → T)	4
			Nonidentified mutation	1
	22	African	29 (A → G)	8
			IVS-II-1 (G → A)	5
			CD 39 (C → T)	3
			IVS-I-110 (G → A)	2
			−88 (C → T)	2
			Initiation CD (ATG → ACG)	1
			Poly A (T → C)	1
	1	Asian	CD 14/15 (+G)	1
Hb C-β-thal	1	African	CD 39 (C → T)	1
β-Thal major	1 (patient 1)	European	IVSI-6 (T → C)/CD 39 (C → T)	
	1 (patient 2)	European	Poly A (T → C)/nonidentified mutation	
	1 (patient 3)	Asian/European	CD 41/42 (-TTCT)/nonidentified mutation	
	1 (patient 4)	Asian/African	CD 41/42 (-TTCT)/-29 (A → G)	
	1 (patient 5)	African	IVS-I-5 (G → A)/IVS-I, -1 (G → A) (CD 30)	
	1 (patient 6)	African	IVS-II-1 (G → A)/nonidentified mutation	
	1 (patient 7)	African	IVS-I-110 (A → G)/poly A (T → C)	

[a]Number of alleles.
[b]Novel mutation.

activation of the cryptic branch site with the branch point located at position −22, since the normal branch site is intact, and the above mentioned in vitro assays showed a clear preference for the −37 branch site [26].

To test these hypotheses, we calculated consensus values (CVs) for the normal (CVN_{-37}) and the potentially activated cryptic branch sites due to IVS-I-108 (T → C) mutation (CVM_{-24} and CVM_{-22}), as well as for the previously described cryptic branch sites with the same branch point (CVC_{-24} and CVC_{-22}). CVs were calculated not only for the heptanucleotide spanning positions −5 to +1 with respect to the branch point nucleotide (taken to be at position 0), but also for sequences spanning positions −5 to +15, associating part of the polypyrimidine stretch with the branch site (see Methods). This association is consistent with the experimental observation that this stretch plays a significant role in determining the efficiency of lariat formation [19]. The potential for cryptic branch site utilization (PCU) was also calculated. From the data presented in Table III, we conclude that an activation of the cryptic branch site with the branch point located at −24 due to IVS-I-108 (T → C) mutation is supported by the CVM_{-24} and PCU values obtained: the values are higher than those obtained for the previously described cryptic branch site with the same branch point (CVC_{-24}); and although the CVM_{-24} scoring positions −5 to +1 is lower than CVN_{-37}, the CVM_{-24} scoring positions −5 to +15 is higher than CVN_{-37}. On the contrary, the activation of a cryptic branch site with the branch point located at −22 due to IVS-I-108 (T → C) mutation is not probable since CVM_{-22} are too low (0.246; 0.437) and comparable with those obtained in the wild type sequence with the branch point at −22 (0.254; 0.440).

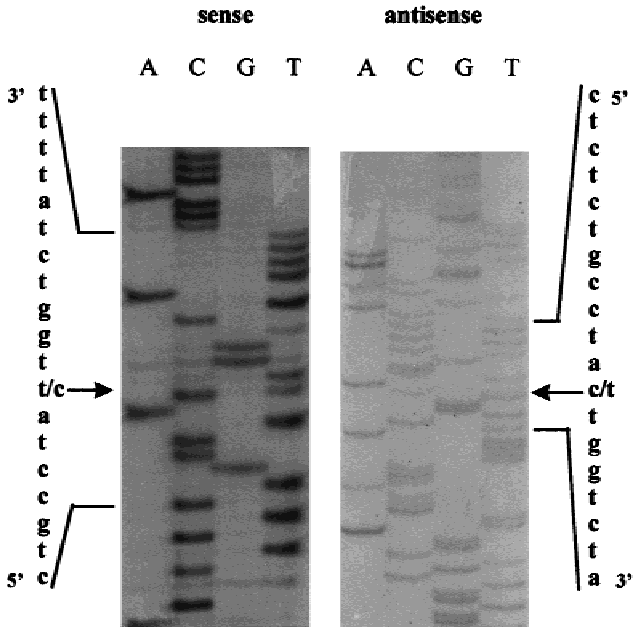

Fig. 1. Direct sequencing leading to the identification of the novel β-globin gene mutation IVS-I-108 (T → C). Sense strand: amplification with the primers 5′-TAAGCCAGTGCCAGAAGAGC-3′ and 5′-TCCCATTCTAAACTGTACCC-3′; sequencing with primer 5′-ATCAAGGTTACAAGACAGG-3′. Antisense strand: amplification with primers 5′-GGTTTCTGATAGGCACTGAC-3′ and 5′-CCTATGACAGAACTTAACC-3′; sequencing with 5′-CCTATGACATGAACTTAACC-3′.

Statistical Analysis

The chi-square (χ^2) statistics was used to assess difference significance in β-thal allele distribution between the Cuban and other related populations (Table IV) and within Cuban subpopulations. European-Cubans were compared with Spaniards, and African-Cubans were compared with both American-Blacks and Guadeloupeans, taking into account the relevant allele classes only (see footnotes in Table IV). Intrapopulation heterogeneity analysis revealed a significant difference between European-Cubans and African-Cubans ($\chi^2 = 17.26$; $df = 4$; $0.001 < P < 0.01$).

DISCUSSION

Mutation Analysis

In order to offer genetic counselling and prenatal diagnosis of β-thal and Hb S-β-thal to at-risk couples, we have delineated the spectrum and relative abundance of β-thal alleles in the Cuban population. Seventeen different point mutations were identified accounting for 93% of the β-thal alleles studied, revealing a high genetic heterogeneity in the Cuban population at the *HBB* locus. All but one of these β-thal mutations had been originally described in subjects from Europe (mainly from Mediterranean countries), Africa (mostly in American-Blacks), or Asia (Chinese). Their co-existence in the Cuban population is consistent with a historical record of high population admixture. In fact, the more prevalent mutations, CD 39 (C → T) (30.5%), −29 (A → G) (13.4%), IVS-I-110 (G → A) (8.5%), and IVS-II-1 (G → A) (8.5%), which account for 61% of the molecular defects, reflect the predominant Mediterranean and African ancestry in Cubans. Surprisingly, given the present ethnic composition of the Cuban population (see above), the next most abundant mutation, CD 41/42 (−TTCT) (6.1%), was described first and is common among Chinese.

In this study, six β-thal alleles were not identified, three of them due to incomplete DNA analysis. In the remaining three, two β-thal carriers and one Hb S-β-thal patient, complete β-globin gene analysis was performed. The nonidentification of these alleles can be owed either to β-globin gene deletions not detected by DGGE, or to β-thal determinants unlinked to the β-globin gene [27].

Novel Mutation

We report the identification of a novel allele, IVS-I-108 (T → C), found in a β-thal carrier of European origin, with no other molecular defect in the β-globin gene (Fig. 1). We hypothesize that this mutation reduces splicing efficiency due to the activation of a cryptic branch site with the branch point at position −24. The usage of the cryptic branch site −24 brings the 3′-splice site thirteen nucleotides closer than in the wild type. This vicinity might result in steric hindrance among the factors which bind RNA to form the spliceosome, causing a diminished splicing efficiency. Another possible explanation is that the new and the authentic branch site lie so close to each other, that there may be interference between the two sequences, competing for the splicing machinery involved in lariat formation, thus diminishing the overall splicing efficiency.

RNA analyses (including in vitro splicing assays), clearly out of the scope in this study, should be performed in order to clarify the functional effect of this nucleotide change which was not found in 64 normal β-globin genes, meaning it is not a common polymorphism.

Comparison Between Cubans and Other Related Populations

Interpopulation heterogeneity tests indicate that the Cuban population as a whole is significantly different from related populations, namely Spaniards, American-Blacks, and Guadeloupeans (Table IV). However, when the frequency of the most common β-globin mutations in the Spanish population [28; M. Baiget, personal communication] was compared with their frequency in Euro-

TABLE III. IVS-I Branch Site Comparison Between the Normal and the Mutant IVS-I-108 (T → C) β-Globin Gene*

Nucleotides spanning positions −5 to +1 with respect to the branchpoint at position 0	Consensus branch site Py_{72} −5	N −4	Py_{86} −3	Py_{72} −2	Pu_{69} −1	A_{86} 0	Py_{69} +1	CV[b]	PCU[c]	CV[d]	PCU[c]
β-Globin IVS-I branch site	***C***	***A***	***C***	***U***	***G***	***A***	***C***	CVN_{-37}		CVN_{-37}	
Branch point →						−37		0.735		0.706	
Activation of a cryptic branch site due to IVS-I-108 (T → C) mutation	***U***	***G***	***C***	***C***	U	***A***	***C***[a]	CVM_{-24}		CVM_{-24}	
Branch point →						−24		0.626	0.85	0.749	1.06
Cryptic branch site activated by an A → G at −37 described in in vitro assays	***U***	***G***	***C***	***C***	U	***A***	***U***	CVC_{-24}		CVC_{-24}	
Branch point →						−24		0.555	0.76	0.721	1.02
Cryptic branch site described in in vitro assays	***C***	***C***	***U***	A	U	U	G	CVC_{-22}		CVC_{-22}	
Branch point →						−22		0.254		0.440	
Activation of the cryptic branch site with the branch point located at −22 due to IVS-I-108 (T → C) mutation	***C***	***C***	***U***	A	C[a]	U	G	CVM_{-22}		CVM_{-22}	
Branch point →						−22		0.246	0.97	0.437	0.99

*Nucleotides in boldface italic type. match with the consensus sequence.
[a]Mutated nucleotide. Py, pyrimidine; Pu, purine; A, adenosine; N, any nucleotide; the numbers in subscript correspond to the probability (%) of occurrence according to the frequency data of Penotti (1991) [19].
[b]Shapiro–Senapathy consensus values calculated by scoring nucleotides spanning positions −5 to +1 with respect to the branch point at position 0.
[c]Potential for cryptic branch site utilization.
[d]Shapiro–Senapathy consensus values calculated by scoring nucleotides spanning poistions −5 to +15 with respect to the branch point at position 0.

TABLE IV. Interpopulation Heterogeneity Tests of β-Thal Allele Frequencies

	Spaniards		American-Blacks	Guadeloupeans
	Benito et al., 1996 [28] (n = 220)[a]	M. Baiget, personal communcation (n = 254)[b]	Gonzalez-Redondo et al., 1991 [30] (n = 128)	Romana et al., 1996 [29] (n = 63)
European-Cubans (n = 36)	NS $P > 0.05$ $\chi^2 = 7.733$ $df = 4$[c]	NS $P > 0.05$ $\chi^2 = 0.764$ $df = 4$[c]		
African-Cubans (n = 38)			S $P < 0.001$ $\chi^2 = 29.65$ $df = 2$[d]	S $0.01 < P < 0.05$ $\chi^2 = 7.878$ $df = 3$[e]
Cubans (n = 82)	S $P < 0.001$ $\chi^2 = 51.33$ $df = 4$[c]	S $P < 0.001$ $\chi^2 = 24.85$ $df = 4$[c]	S $P < 0.001$ $\chi^2 = 96.49$ $df = 3$[f]	S $P < 0.001$ $\chi^2 = 40.59$ $df = 4$[g]

S, significant difference; NS, nonsignificant difference; *df*, degrees of freedom; *n*, number of alleles.
[a]Comprises alleles from Huelva (18), Cáceres (75), Granada (45), Barcelona (58), and Madrid (24) provinces.
[b]Comprises alleles from all over Spain: CD39 (C → T) (120), IVS-I-110 (A → G) (28), IVS-I-1 (G → A) (22), IVS-I-6 (T → C) (24), other mutations (60).
[c]Allele comparison: CD39 (C → T); IVS-I-110 (A → G); IVS-I-1 (G → A); IVS-I-6 (T → C); other mutations.
[d]Allele comparison: −29 (A → G); −88 (C → T); other mutations.
[e]Allele comparison: −29 (A → G); IVS-I-5 (G → A); IVS-II-1 (G → A); other mutations.
[f]Allele comparison: −29 (A → G); −88 (C → T); CD39 (C → T); other mutations.
[g]Allele comparison: −29 (A → G); IVS-I-5 (G → A); IVS-II-I (G → A); CD39 (C → T); other mutations.

pean-Cubans, no statistically significant difference was found. So, we can say that the four more common Mediterranean β-thal mutations (CD 39 (C → T), IVS-I-110 (G → A), IVS-I-1 (G → A), and IVS-I-6 (T → C)) which account for 75% of β-thal alleles among European-Cubans, occur in proportions that strictly parallel those observed in the Spanish population. We suggest that the discrepancy observed in the χ^2 value obtained in the comparison of European-Cubans with the two Spanish population samples might be due to the enrichment of the Spanish population sample studied by Benito et al. [28] with alleles from Southwestern Spain, where IVS-I-1 (G →

A) reaches the highest known frequency (the prevalence of this mutation in Spain varies greatly among the different provinces, ranging from 3% in Barcelona to 55% in Huelva). Globally, the two populations are significantly different ($P < 0.001$), essentially due to the Cuban admixture of Spaniards and sub-Saharan Africans, who have a completely different pool of β-thal mutations.

Allele frequency comparisons between African-Cubans and Guadeloupeans [29] indicate a borderline significant difference ($0.01 < P < 0.05$). This borderline result, may be explained by the fact that these two populations have two African β-thal mutations (−29 (A → G) and IVS-II-1 (G → A)) in common, and have undergone a similar dilution effect in the frequency of these mutations due to population admixture. Globally, the Cuban and Guadeloupean populations are significantly different ($P < 0.001$), reflecting the sub-Saharan admixture, more pronounced in Cubans, with two different Caucasian populations (Spanish in Cuba; French, Syrians, and Lebanese in Guadeloupe) and two different Asian populations (Chinese in Cuba; Asian Indians in Guadeloupe).

The African-Cuban sub-population is highly significantly different ($P < 0.001$) from the American-Black population [30]. In the latter, two African mutations −29 (A → G) and −88 (C → T) account for 81% for the β-thal mutations, whereas in the former they only represent 32% of the molecular defects. This observation can be explained by the fact that American-Blacks have been kept, until recently in history, an isolated population by racial barriers.

Feasibility of Prenatal Diagnosis (PND)

With the mutation screening strategy applied in this study, 93% of β-thal carriers could be characterized at the molecular level. Therefore, this approach will allow the offering of fully informative β-thal PND to nearly 87% of couples at-risk. For Hb S-β-thal this figure rises to 93%.

A viable, albeit less effective, alternative method of diagnosis, could be the use of ARMS or reverse dot-blot, detecting eight of the more frequent mutations in the Cuban population: CD 39 (C → T), −29 (A → G), IVS-I-110 (G → A), IVS-II-1 (G → A), CD 41/42 (−TTCT), IVS-I-1 (G → A), IVS-I-6 (T → C), and −88 (C → T). With this method one should be able to identify nearly 78% of the β-thal mutations and therefore offer PND to 61% and 78% of couples at-risk for β-thal or Hb S-β-thal, respectively. Due to the close association (76% concordance) between specific mutations and the geographic origin of the three Cuban subpopulations, sequential mutation analysis should be performed.

Given the mutation spectrum above, disease severity prediction within the context of prenatal diagnosis may be difficult in some instances, specially whenever two mild β-thal alleles (which account for about 25% of all alleles) are found in a fetus. In order to help solve this counseling problem, a continuous effort should be put in correlating clinical and genetic data of all diagnosed β-thal and Hb S-β-thal patients. In addition to the β-globin genotyping, the genetic characterization should include β-gene cluster haplotypes and α-gene cluster status, two well-known disease severity modulators.

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